PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P108		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/03605	20/09/2000	20/09/1999
International Patent Classification (IPC) or national classification and IPC C12N15/13		
Applicant ABERDEEN UNIVERSITY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/04/2001	12.12.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Dumont, E Telephone No. +49 89 2399 7704

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03605

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-20 as originally filed

Claims, No.:

1-20 with telefax of 21/11/2001

Drawings, sheets:

1/2,2/2 as originally filed

Sequence listing part of the description, pages:

1,2, filed with the letter of 06.10.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 19.

because:

- ☒ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
  see separate sheet

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

- ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-20
Industrial applicability (IA)	Yes:	Claims	1-18, 20
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following document cited in the International Search Report (ISR):
D1:US-A-5 614 611

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 19 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application relates to a pharmaceutical composition comprising a nucleic acid construct which encodes a recombinant antibody molecule against a "disease-causing agent", i.e. a pathogen, an allergen or a toxin. Preferably the encoded antibody is a single-chain antibody comprising variable domains of immunoglobulin Heavy and Light chains connected by a linker sequence. The nucleic acid construct can further encode a secretion signal peptide. The pharmaceutical composition can be administered to animals for *in vivo* production of antibody molecules and subsequent establishment of protective immunity. More specifically, the application discloses a construct encoding a viral haemorrhagic septicaemia virus (VHSV)-neutralizing antibody, 3F1H10, with two amino acid substitutions in the H-chain. This construct comprises a single chain antibody gene (BU1) encoding the variable domains of mutated 3F1H10 fused to a signal peptide and inserted into the pCDNA3 plasmid under control of the CMV promoter. Establishment of protective immunity against VHSV upon injection of pCDNA3-BU1 to fish was demonstrated.

2. Novelty (Art. 33(2) PCT)

With regard to the result of the ISR, the subject-matter of claims 1-20 appears to be novel: although compositions comprising nucleic acid constructs encoding recombinant antibodies are known, the cited prior art does not disclose such a composition for use in

therapy practiced on the human or animal body (first medical use).

3. Inventive step (Art. 33(3) PCT)

-The subject-matter of claims 1-20 appears to lack an inventive step in view of D1 in combination with the common knowledge of the skilled person. *In vivo* administration of free antibodies is described in D1 (col. 4, line 44 - col.5, line 11). Furthermore, the use of antigen-encoding DNAs as "vaccines" is known to the skilled person. The administration of plasmid DNA encoding an antibody, in order to provide an alternative way of passive immunization, therefore merely consists in the use of a known technique in a closely analogous situation and is thus not considered to involve an inventive step.

-In the absence of any specific advantage or unexpected technical effect of the mutated VHSV-neutralizing antibody 3F1H10 over the antibodies available from the prior art, the IPEA fails to see any further inventive contribution in a composition comprising this specific antibody (also see remarks to item VIII, 2.).

4. Industrial applicability

The attention of the applicant is drawn to the fact that the subject-matter of claim 19 is directed to methods of treatment of the human or animal body and thus, it may be excluded from examination by Article 34(4)(a)(i) PCT in combination with Rule 67(iv) PCT. Furthermore, for such a subject-matter no unified criteria exist in the PCT Contracting States for the assessment whether it is industrially applicable or not. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

Certain defects in the international application

A document reflecting the prior art described on page 1, regarding passive immunization, *in vitro* production of antibodies and their use, is not identified in the description. A prior art document describing antibody 3F1H10 is also missing (Rule 5.1(a)(ii) PCT).

Re Item VIII

Certain observations on the international application

Clarity of the claims (Art. 6 PCT)

1. The term "nucleic acid" renders the scope of claim 1 broader than justified by the description, since the claimed invention is described only for DNA constructs, whereas the use of RNA constructs, which are known to be unstable, is not demonstrated (Art. 6 in combination with Art. 5 PCT).

2. Claim 14 lacks support from the description, since neither antibody 3F1H10, nor its variant having two amino acid substitutions, are characterized. In the absence of data, the selection of mutated 3F1H10 in the claimed invention appears to be arbitrary.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

ABLETT ,Graham Keith
ABLETT & STEBBING
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101-103 Baker Street
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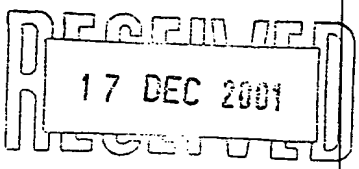

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	12.12.2001
Applicant's or agent's file reference P108	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/03605	20/09/2000	20/09/1999

Applicant
ABERDEEN UNIVERSITY et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hingel, W Tel.+49 89 2399-8717

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P108		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/03605	20/09/2000	20/09/1999
International Patent Classification (IPC) or national classification and IPC C12N15/13		
Applicant ABERDEEN UNIVERSITY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/04/2001	12.12.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Dumont, E Telephone No. +49 89 2399 7704

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03605

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-20 as originally filed

Claims, No.:

1-20 with telefax of 21/11/2001

Drawings, sheets:

1/2,2/2 as originally filed

Sequence listing part of the description, pages:

1,2, filed with the letter of 06.10.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 19.

because:

   ☒ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-20
Industrial applicability (IA)	Yes:	Claims	1-18, 20
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following document cited in the International Search Report (ISR):
D1:US-A-5 614 611

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 19 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application relates to a pharmaceutical composition comprising a nucleic acid construct which encodes a recombinant antibody molecule against a "disease-causing agent", i.e. a pathogen, an allergen or a toxin. Preferably the encoded antibody is a single-chain antibody comprising variable domains of immunoglobulin Heavy and Light chains connected by a linker sequence. The nucleic acid construct can further encode a secretion signal peptide. The pharmaceutical composition can be administered to animals for *in vivo* production of antibody molecules and subsequent establishment of protective immunity. More specifically, the application discloses a construct encoding a viral haemorrhagic septicaemia virus (VHSV)-neutralizing antibody, 3F1H10, with two amino acid substitutions in the H-chain. This construct comprises a single chain antibody gene (BU1) encoding the variable domains of mutated 3F1H10 fused to a signal peptide and inserted into the pCDNA3 plasmid under control of the CMV promoter. Establishment of protective immunity against VHSV upon injection of pCDNA3-BU1 to fish was demonstrated.

2. Novelty (Art. 33(2) PCT)

With regard to the result of the ISR, the subject-matter of claims 1-20 appears to be novel: although compositions comprising nucleic acid constructs encoding recombinant antibodies are known, the cited prior art does not disclose such a composition for use in

therapy practiced on the human or animal body (first medical use).

3. Inventive step (Art. 33(3) PCT)

-The subject-matter of claims 1-20 appears to lack an inventive step in view of D1 in combination with the common knowledge of the skilled person. *In vivo* administration of free antibodies is described in D1 (col. 4, line 44 - col.5, line 11). Furthermore, the use of antigen-encoding DNAs as "vaccines" is known to the skilled person. The administration of plasmid DNA encoding an antibody, in order to provide an alternative way of passive immunization, therefore merely consists in the use of a known technique in a closely analogous situation and is thus not considered to involve an inventive step.

-In the absence of any specific advantage or unexpected technical effect of the mutated VHSV-neutralizing antibody 3F1H10 over the antibodies available from the prior art, the IPEA fails to see any further inventive contribution in a composition comprising this specific antibody (also see remarks to item VIII, 2.).

4. Industrial applicability

The attention of the applicant is drawn to the fact that the subject-matter of claim 19 is directed to methods of treatment of the human or animal body and thus, it may be excluded from examination by Article 34(4)(a)(i) PCT in combination with Rule 67(iv) PCT. Furthermore, for such a subject-matter no unified criteria exist in the PCT Contracting States for the assessment whether it is industrially applicable or not. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

Certain defects in the international application

A document reflecting the prior art described on page 1, regarding passive immunization, *in vitro* production of antibodies and their use, is not identified in the description. A prior art document describing antibody 3F1H10 is also missing (Rule 5.1(a)(ii) PCT).

Re Item VIII

Certain observations on the international application

Clarity of the claims (Art. 6 PCT)

1. The term "nucleic acid" renders the scope of claim 1 broader than justified by the description, since the claimed invention is described only for DNA constructs, whereas the use of RNA constructs, which are known to be unstable, is not demonstrated (Art. 6 in combination with Art. 5 PCT).

2. Claim 14 lacks support from the description, since neither antibody 3F1H10, nor its variant having two amino acid substitutions, are characterized. In the absence of data, the selection of mutated 3F1H10 in the claimed invention appears to be arbitrary.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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29 March 2001 (29.03.2001)

PCT

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WO 01/21800 A1

(51) International Patent Classification[7]: C12N 15/13, C07K 16/08, 16/42, A61K 39/395, A61P 37/08, 31/00

(21) International Application Number: PCT/GB00/03605

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20 September 2000 (20.09.2000)

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(26) Publication Language: English

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PA 1999 01329 20 September 1999 (20.09.1999) DK

(71) Applicants *(for all designated States except US)*: **ABERDEEN UNIVERSITY** [GB/GB]; Auris Business Centre, 23 St. Machar Drive, Aberdeen AB2 1RY (GB). **STATENS VETERINÆRE SERUMLABORATORIUM** [DK/DK]; Hangøvej 2, DK-8200 Århus N (DK).

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(75) Inventors/Applicants *(for US only)*: **SECOMBES, Christopher, John** [GB/GB]; 22 Old Mill Crescent, Balmedie, Aberdeenshire AB23 8WA (GB). **CUNNINGHAM, Charles** [GB/NO]; Parkveien 4B, N-5007 Bergen (NO). **LORENZEN, Niels** [DK/DK]; Vadsmøllevej 27, DK-8350 Hundslund (DK).

(74) Agents: **ABLETT, Graham, Keith** et al.; Ablett & Stebbing, Caparo House, 101-103 Baker Street, London W1M 1FD (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/21800 A1

(54) Title: MONOCLONAL ANTIBODY 3F1H10 NEUTRALISING VHSV (VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS)

(57) Abstract: The present invention relates to a non-infectious nucleic acid (RNA and DNA) construct constructed to express a recombinant antibody or antibody fragment in a host cell. The antibody molecule confers protection to the host against a pathogen, allergen or toxin. The host may be any animal including a human.

MONOCLONAL ANTIBODY 3F1H10 NEUTRALISING VHSV (VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS)

The present invention relates to a non-infectious nucleic acid (RNA and DNA) construct constructed to express a recombinant antibody or antibody fragment in a host cell. The antibody molecule confers protection to the host against a pathogen, allergen or toxin. The host may be any animal including a human.

Passive immunization by injection of homologous or heterologous serum-antibodies is routinely used in humans for immunoprophylaxis of people traveling to foreign regions involving risk of exposure to exotic pathogens. In animals a similar strategy may be employed for protection of valuable specimens, but is generally too expensive for routine veterinary use. Passive immunisation of animals against infectious diseases is thus mostly done on an experimental basis with the aim of studying the function of structures such as antibodies *in vivo* and relating the results to *in vitro* experiments.

During the recent decade, diverse technologies for the *in vitro* production of antibodies by the use of recombinant DNA technology has been developed. The smallest functional recombinant antibody combining the actions of the heavy (H) and light (L) polypeptide chains as in the native molecule has proved to be the single chain variable-fragment construct (single chain FV). The single chain FV construct is composed of the variable parts of the H and L chains connected by a flexible spacer region. Such molecules have been used in various studies including virus neutralisation, cancer-immunotherapy and recently also in the form of DNA vaccines where plasmids encoding anti-idiotype single chain FV

antibodies have proved able to induce an antigen-specific immune response. However, direct establishment of protective immunity to infectious diseases by prophylactic treatment with plasmid DNA carrying single chain FV genes encoding protective antibodies has not been described.

An object of the present invention is to provide a non-infectious nucleic acid construct which can produce an antibody molecule *in vivo* thereby conferring immunity to a disease.

A further object of the present invention is to provide a method of establishing immunity against a pathogen.

A yet further object of the present invention is to provide a method of therapy for animals which have a deficient immune system.

An additional object of the present invention is to provide a method of therapy for an animal suffering from an allergic reaction or a method of preventing an allergic reaction.

For avoidance of doubt it should be noted that the word "animal" includes but is not restricted to mammals including humans.

According to an embodiment of the present invention there is provided a nucleic acid construct encoding a recombinant antibody molecule, said construct being adapted for the *in vivo* establishment of a protective immunity to an infectious disease in an animal.

According to a further embodiment of the present invention there is provided a nucleic acid construct encoding a recombinant antibody molecule, said construct is formulated for the *in vivo* prevention of an allergic reaction to an allergen in an animal.

According to a yet further embodiment of the present invention there is provided a nucleic acid construct encoding a recombinant antibody molecule, wherein said construct is formulated for the *in vivo* prevention of a reaction caused by the presence of a toxic substance in an animal.

The term recombinant antibody molecule encompasses a full size antibody, a single chain variable fragment or any part of an antibody which can recognise an antigen. In this connection, conveniently the antibody fragment does not have to be single chain. However, in some embodiments it is single chain.

It has now been found that the intramuscular injection of a nucleic acid construct, in the form of a plasmid, encoding a virus-neutralising single chain antibody fragment can mediate *in vivo* expression of antibodies which protect an animal against a possibly lethal exposure to a virus. This has been established in an experimental model which involves a fish rhabdovirus called viral haemorrhagic septicaemia virus (VHSV) in the rainbow trout (Oncorhynchus mykiss) as a host species.

According to a further embodiment of the present invention there is provided a nucleic acid construct, such as a plasmid, comprising an expression vector and a gene sequence for heavy and/or light chain variable domains of an antibody.

Preferably the heavy and light chain variable domains are linked by a linker sequence in order that they form what is known in the art as a single chain variable-fragment.

It is thought that the antibody fragment as expressed in and secreted from a host cell carrying the vector will act with the same specificity as a natural antibody would in the presence of a substance which it recognises. In this connection, for example, if the heavy and/or light chain variable domain were derived from a monoclonal antibody raised against dengue virus then if dengue virus infected a host who had received a nucleic construct expressing a single chain variable fragment produced from the heavy and light chain of the monoclonal antibody, the fragment would recognise cells infected with the dengue virus or the dengue virus particle itself and bind thereto thereby neutralising or inhibiting the virus and/or giving the host time to mount an immune response against the virus.

In preferred embodiments the expression vector is made for eukaryotic expression and/or is non infectious. For example, a bacterial plasmid, or a smaller DNA fragment carrying the variable fragment antibody gene within a eukaryotic expression operon including regulatory elements such as an enhancer, promoter and polyadenylation signal could be used. Alternatively, stabilised messenger RNA including a positive strand transcript of the variable-fragment antibody gene with translation signals may be employed.

The antibody fragment genes can be cloned by any method known to those skilled in the art, for example from hybridoma cells or directly from B-lymphocytes from immunized individuals. Nucleic acid constructs encoding protective antibody fragments

can be prepared against any important pathogen/disease causing agent in animals including pathogens against which vaccines are not available or have proved insufficient. Furthermore, as a result of veterinary regulations, use of live vaccines may not be allowed. In such cases an alternative prophylactic measure would have to be taken. Such a measure could be the administration of the nucleic acid construct of the present invention. A list of possible pathogens is given below; this list is not intended to be exhaustive.

Viral haemorrhagic septicaemia virus (fish)
Infectious haematopoietic necrosis virus (fish)
Infectious salmon anemia virus (fish)
Infectious pancreatic necrosis virus (fish)
Nodaviruses (fish)
Renibacterium salmoniarum (fish)
Pasteurella (fish)
Ichthyopthtirius mulitifiliis (fish)
NewCastle disease virus (fowl)
Infectious bursal disease virus(fowl)
Bovine respiratory syncytial virus (cattle)
Bovine virus diarrhoea virus (cattle)
Porcine reproductive and respiratory syndrome virus (pigs)
Pseudorabiesvirus (pigs)
Equine herpes virus 1 (horses)
Plasmocytosis virus (mink)
Rabies virus (dogs)
Feline leukemia virus (cats)
Foot and mouth disease (cattle)
Human immune deficiency virus (human)
Hepatitis A virus (human)
Borrelia sp. (human)
Plasmodium sp. (human)

Rabies virus (human)
Epstein-Barr virus (human)

In case of humans with either a congenital or acquired immunodeficiency, vaccines will generally be insufficient. In such cases, administration of a number of nucleic acid constructs according to the present invention encoding antibodies against a broad spectrum of pathogens may be considered.

For the purpose of prevention of allergic relations induced by IgE response, administration of nucleic acid constructs mediating expression of an allergen-specific recombinant antibody may be used to competitively inhibit binding of the allergen to the IgE molecules in the host. Alternatively gene constructs encoding anti-IgE antibodies may be used to interfere with the interaction between IgE and mast cells in the allergic individual.

Administration of antibody gene constructs encoding antibodies to toxins or venoms can be used for the prophylactic treatment of individuals periodically being in high risk of exposure to toxic organisms. The venoms could, for example, be from snakes or spiders.

Conveniently the construct further comprises a gene encoding a signal sequence for the secretion of the product encoded by the gene sequence. The signal sequence will allow the product of the gene sequence to be secreted from a cell in which the gene has been expressed, into the blood so that the product of the gene sequence can circulate therein. For example, the genes for the signal sequence of either rainbow trout transforming growth factor beta (TGF-beta), or murine Ig

kappa-chain can be added to the 5' end of a gene to be administered to the fish. Other secretion signals, preferably of homologous origin to the host species may be employed. Examples of genes which encode proteins which act as secretion signals include the gene for immunoglobulin heavy and light chain secretion signals or other glycoprotein secretion signals. Preferably, the secretion signal should include a proteolytic cleavage site ensuring removal of the signal peptide before secretion of the antibody fragment.

Preferably the construct further comprises a known gene sequence which encodes a short peptide sequence that can be used to identify transfected cells. Such a gene sequence can be attached to the 3' end of the gene. Examples of such a sequence include a human kappa light chain construct or sequence encoding a six histidine residue. In both cases, an antibody specifically recognising the expressed peptide is commercially available.

The construct according to the present invention may be delivered by any suitable method, such as by injection (e.g intramuscularly), by a spray on a mucosa surface (e.g intranasally), by particle bombardment on skin/dermis through use of a gene gun, by electroporation or by uptake by an animal from an aqueous environment. In this connection, the plasmid may be encased in a liposome for administration to an animal. The construct may be administered to the animal topically, through inhalation or orally. For oral administration the construct should be protected from degradation by proper encapsulation.

It is preferred that in a composition or formulation for administration of the constructs there are present genes

encoding the heavy and/or light chain variable fragments against several different epitopes or an variable fragment antibody gene expression library against a given pathogen. In this connection, the various fragments may be provided on one plasmid or they may be provided on several different gene constructs which are all present in the same formulation or other method of administration. In the alternative, each plasmid may have to be administered separately.

The invention also provides for a method for treating an animal, for example a mammal or a fish which comprises administering thereto a plasmid or other nucleic acid construct encoding a protective antibody fragment as previously described.

The invention thus provides for a method of therapy for an animal which has a deficient immune system.

The invention also provides for a therapeutic composition comprising the plasmid as previously described and a pharmaceutically acceptable diluent or carrier therefor. The composition may be formulated such that it is in the form of, for example, a vaccine, dosage form, cream, ointment, liquid or paint.

The invention will now be described by way of illustration only with reference to the following Example and Figures.

Figure 1 shows a schematic drawing of the pCDNA3 plasmid with a single chain antibody (ScAb) gene construct inserted downstream of a strong eukaryotic promoter from cytomegalovirus (CMV). PCDNA3 is a commercially available eukaryotic expression vector (Invitrogen).

Figure 2 shows a culture of EPC cells (passaged fish cells) transfected with a pCDNA3-BU1. BU1 is a ScAb gene construct encoding a recombinant antibody which is able to neutralise the fish pathogenic rhabdovirus, VHSV. BU1 carries a part of the human kappa light chain gene as a residue or tag. Twelve days after the date of transfection the cells were fixed and stained immunochemically using horseradish peroxidase-conjugated rabbit antibody to human kappa light chain (HRP-Rabbit anti-kappa) for the detection of cells containing ScAb. These cells give a positive response and are darker than the remaining cells; and

Figure 3 shows a histological section of muscle tissue sampled from a fish twelve days after intramuscular injection of pCDNA3-BU1. The section was stained immunochemically using HRP-rabbit anti-kappa for the detection of ScAb. Several cells turned out positive (arrow heads) along the regenerating needle track (injection site) arrowed.

Gene Map

The following gene map is the DNA sequence of the construct comprising a single chain antibody gene (BU1) inserted into E.coli pCDNA3 plasmid (Invitrogen) used in the Example described below.

1 cagtgtgcta acatgagggc agtgtgtttg atgctgactg ccttattgat
51 gctggaatat gtgtgccgga gtgaccaggt gcagctgcag gagtcaggac
101 ctggcctcgt gaaaccttct cagtctctgt ctctcacctg ctctgtcact
151 ggctactcca tcaccagtgg ttattactgg acctggatcc ggcagtttcc
201 aggaaataaa ctggaatgga tgggctacat aagctacgac ggtaccaata
251 actacaaccc atctctcaca aatcgaatct ccatcactcg tgacacatct
301 aagaaccagt ttttcctgaa gttgaaatct gtgactactg aggacacagc

351 tacatattac tgtgtaagag ggatctacta tggtaacgac tggtttgctt
401 actggggcca agggaccacg gtcaccgtct cctcagaagg caaatcttct
451 ggctctggct ctgaatctaa agtggatgac atcgagctca cccagtctcc
501 tgcctcccag tctgcatctc tgggagaaag tgtcaccatc acatgcctgg
551 caagtcagac cattggtaca tggttagcat ggtatcaaca gaaaccaggg
601 aaatctcctc agctcctgat ttatgctgca accagtttgg cagatggggt
651 cccatcaagg ttcagtggta gtggatctgg cacaaaattt tctttcaaga
701 tcagcagcct acaggctgaa gattttgtaa gttattactg tcaacaactt
751 tacagtactc cgtacacgtt cggaggggg accaagctcg agatcaaacg
801 gactgtggct gcaccatctg tcttcatctt cccgccatct gatgagcagt
851 tgaaatctgg aactgcctct gttgtgtgcc tgctgaataa cttctatccc
901 agagaggcca aagtacagtg gaaggtggat aacgccctcc aatcgggtaa
951 ctcccaggag agtgtcacag agcaggacag caaggacagc acctacagcc
1001 tcagcagcac cctgacgctg agcaaagcag actacgagaa acacaaagtc
1051 tacgcctgcg aagtcaccca tcagggcctg agttcgcccg tcacaaagag
1101 cttcaaccgc ggagagtcat aagttagata tccat

The BU1 insert (ScAb gene construct) is encoded by nucleotides 10 to 1125. The coding region nucleotides are 13 to 1122.

The above identified sequence can be found in the Genebank, the Accession Number is AF302092.

Example

Single chain antibody genes were prepared according to the procedure described by McGregor et al; Spontaneous Assembly of Divalent Single Chain Antibody Fragments in E-Coli; Mol. Immunol, February 31(3) pp 219 to 226; 1994. In short, the variable domains of the immunoglobulin H and L chain genes were cloned from hybridoma cell lines producing monoclonal antibodies to the fish pathogenic rhabdovirus viral haemorrhagic septicaemia virus(VHSV). The H and L chain variable domains were linked by a gene sequence encoding a 14

amino acid linker to generate a single chain antibody (ScAb) gene. As a tag to allow specific detection, the human kappa light chain constant domain gene was included at the 3' end of the gene. In order to ensure secretion of the ScAb polypeptides in eukaryotic cells, the nucleotide sequence encoding the 20 amino acid signal peptide of rainbow trout transforming growth factor beta (TGF-beta) was added at the 5' end of the gene.

The gene construct was inserted by blunt-end ligation into the eukaryotic expression vector pCDNA3 (Invitrogen) in the EcoR I site in the polylinker downstream of a cytomegalovirus (CMV) promoter (see Figure 1). As a negative control in transfection experiments with cell cultures and immunoprotection trials in fish, the pCDNA3 plasmid without insert was used. Plasmid DNA was purified from overnight cultures of *E.coli* by use of commercial kits for anion-exchange chromatography as recommended by the supplier (Qiagen).

Other molecular biology procedures used were as followed by Sambrook et al in Molecular Cloning; A Laboratory Manual, Second Addition, Cold Spring Harbor Laboratory, USA, (1989). The variable domain genes from a hybridoma cell line secreting the VHSV-neutralising monoclonal antibody 3F1H10 were used. Cloning and sequencing of the variable domain genes has already been described. In the case of antibody 3F1H10, two amino acids substitutions were made to the H-chain (Asn35a to Thr and Lys64 to Thr). The ScAb carrying the variable domains of antibody 3F1H10 was called BU1.

Passaged fish cells designated (EPC) were transfected with an anionic transfection reagent (Superfect, Qiagen). Four to six

days after transfection cell culture supernatant were harvested and analysed for antibody reactivity to VHSV. After removal of the supernatant, the cells remaining attached to the bottom of the cell culture wells were fixed in 80% cold acetone and stained by immuno-peroxidase using horseradish peroxidase-conjugated rabbit antibody to human kappa light chain (HRP-Rabbit anti-kappa)(DAKO, Denmark) in order to detect cells expressing ScAb. The effect of transfection on the susceptibility of the cell cultures to VHSV different doses of live VHSV was examined by adding the different doses to wells with cultures of transfected cells four days after transfection and the development of cytopathogenic effects (CPE) was recorded thereafter.

Injection of Plasmid DNA into Fish

Disease free rainbow trout fingerlings, average weight 4.5g, were anaesthetised with 0.001% benzokaine and given two 25μl injections of 20 μg plasmid DNA each, in the epaxial muscles below the dorsal fin. The fish were afterwards kept in groups of approximately 150 individuals in 120-liter tanks supplied with running tap water. The fish were fed *ad libitum* with commercial fish feed. Mean water temperature was 16°C. Injected plasmid constructs included the pCDNA3 vector without insert, and pCDNA3 carrying the ScAb BU1 gene construct (pCDNA-BU1) respectively.

Immunohistochemical Analysis for Expression of ScAb in Injected Fish

Twelve days after injection of plasmid DNA, 10 fish were sampled for each plasmid construct. After termination of the fish a section of muscle tissue was excised from the site of injection. The tissue was fixed in 10% phosphate buffered formalin and analysed by immunohistochemistry. Horseradish

peroxidase-conjugated rabbit immunoglobulin (Ig) to human kappa light chain (HRP-rabbit anti kappa) (Dako, Denmark) was used for detection of expressed ScAb.

Sampling of Plasma from Fish

Blood samples were collected 12 days after injection of plasmid DNA from fish not exposed to VHSV. Due to the small size of the fish, sampling was performed with heparin-treated capillary tubes after cutting off the posterior fin of fully anaesthetised fish. The fish were terminated immediately afterwards. The blood samples were centrifuged at 5000 xg and plasma samples were collected and stored at -80°C until analysed.

Serological Examination for VHSV-reactive ScAbs

Supernatant from transfected cell cultures and plasma samples from DNA-injected fish, were examined for anti-VHSV reactive ScAbs by a plaque-neutralisation (50% PNT) assay and by an enzyme-linked immunosorbent assay (ELISA).

The ELISA assay was performed in 96-well microtitre plates coated with purified VHSV. Bound ScAb's were detected with HRP-Rabbit anti-kappa. In order to demonstrate that the virus-neutralising activity detected in the trout plasma was due to the ScAbs produced by the fish and not by trout antibodies, two variants of the 50% PNT assay were also applied. One variant included parallel examination of the neutralising activity against the virulent VHSV3592B and a neutralisation resistant variant of VHS 3592B (VHSV DK-3542B) selected by cultivating virus in the presence of the neutralising Mab 3F1A2 which is highly similar to Mab 3F1H10. The other variant involved pre-incubation of the trout plasma with rabbit antibodies to human kappa light chain or with

rabbit antibodies to trout immunoglobulin before incubation with virus. The 50% PNT microplate assay was performed as described by Olesen and Jørgensen in Detection of neutralising antibody to Egtved virus in rainbow trout by plaque neutralising with complement addition, J. Appl Ichthyol, Volume 2, pages 35 to 41.

Immunoprotection Trials in Fish

Eleven days after injection of the plasmid, groups of fish were exposed to (challenged with) the virulent VHSV DK-3592B isolate by immersion in water containing 100 000 50% tissue-culture infective doses per ml. Challenge was performed in 8-liter aquaria with 25-31 fish in each. Three replicate aquaria was included for each plasmid construct. Dead fish were afterwards daily recorded and collected. Dead fish from all tanks were analysed virologically for the presence of VHSV. Mean water temperature was 16°C from the time of injection to immediately before challenge. At challenge, the fish were adapted to a water temperature of 12°C and this temperature was kept throughout the 20 day challenge period.

Immunochemical Detection of Expressed ScAb in cell Culture and in Fish

It was found that after immuno-peroxidase staining using the HRP-rabbit anti-human kappa, single cells expressing ScAb could be detected in EPC cell cultures transfected with the plasmid construct pCDNA3-BU1 (Fig. 2), whereas no positive cells were found in cultures transfected with pCDNA3 without insert. Similarly, expression of ScAb could be demonstrated in muscle sections from injected fish (Fig. 3). No positive cells were found in fish injected with pCDNA3 without insert.

Interference of ScAbs with propagation of VHSV in Cell Culture

When monolayers of epithelial cell line of cap cell cultures were inoculated with VHSV four days after transfection, development of cytopathogenic effect (CPE) as a result of multiplication of VHSV was highly different in cultures transfected with pCDNA3 compared to cell cultures transfected with pCDNA3-BU1. In the latter case only certain plaques of cells became infected and died and there was no further development of CPE in the 8-day observation period. In contrast, when cultures transfected with pCDNA3 were inoculated, all cells became infected and were destroyed within 3-6 days as in a normal propagation of VHSV in EPC cells (Table 1).

Table 1. Susceptibility of transfected EPC cell cultures to VHSV

Plasmid Construct used for Transfection	**Cytopathogenic effect upon inoculation with VHSV***
pCDNA3	Complete destruction of cell layer
pCDNA3-BU1	Plaques

* Concentrations of VHSV: 10^2-10^3 TCID-50/ml cell culture medium.

Detection of ScAbs to VHSV in the Fish

When the plasma from injected fish was analysed by ELISA for ScAbs recognising VHSV, a strong reaction was found in plasma from fish injected with pCDNA3-BU1. No reactivity was detected in plasma from fish injected with pCDNA3 without insert. As indicated in Table 2, the limited amounts of

plasma available made it necessary to perform the analysis on pools of five individuals. The 50% PNT analysis was performed on individual plasma samples. All 10 individuals injected with pCDNA3-BU1 neutralised VHSV, whereas no neutralising activity was detected in plasma from fish injected with the pCDNA3 (Table 3). When plasma from fish injected with pCDNA3-BU1 was preincubated with Rabbit anti-human kappa before testing in 50% PNT, the neutralising activity was eliminated, whereas no effect was observed upon pre-incubation with normal rabbit serum or with rabbit serum to trout Ig (Table 4). The neutralising activity of a positive trout serum control was unaffected by pre-incubation with normal rabbit serum and with rabbit anti-human kappa, but was highly reduced upon pre-incubation with rabbit serum to trout Ig (Table 4). As with the parent monoclonal antibody 3F1H10, plasma samples from fish injected with pCDNA3-BU1 could neutralise the virulent VHSV DK-3592B isolate, but not a neutralisation escape-mutant (not shown).

Table 2. Antibody reactivity in fish plasma: ELISA

Fish No. *	Injected Plasmid	Reactivity with VHSF (A-496 mm)	
		Dilution: 1/10	Dilution: 1/80
36529	pCDNA3	0	0
36686		0	0
36844	pCDNA3-BU1	3	1
16-20		3	1

* The plasma samples were analysed in pools of 5 individuals.

Table 3. Antibody reactivity in fish plasma: Neutralisation of VHSV

Fish No. *	Injected Plasmid	PNT-titres **
36534	pCDNA3	<10
36849	pCDNA3-BU1	160-640

* Plasma samples were analysed individually.

** Titres represent the reciprocal value of plasma dilutions reducing the number of plaques to approximately 50% compared to a control well without antibody/plasma.

Table 4. Effect of preincubation of trout plasma with rabbit antibodies on PNT-titres*

Fish No.	Injected Reagent	PNT-titres		
		Normal rabbit	Rabbit to human chain kappa	Rabbit to trout Ig
21-30 (1 pool)	pCDNA3-BU1	640	<40	320-640
Positive trout serum A7.1	Killed VHSV	>10240	>10240	320

* In order to allow detection of neutralising trout antibodies, trout complement was included as described above.

Infection Trial

When challenged with VHSV DK-3592B 11 days after injection of plasmid DNA, most of the fish injected with pCDNA3-BU1 survived whereas high mortalities were observed among fish injected with pCDNA3 (Table 5).

Table 5. Protection against VHSV

Injected Plasmid	Accumulated mortality 20 days post challenge (mean of triplicate tanks)
pCDNA3	81%
pCDNA3-BU1	6%

To our knowledge, this is the first report demonstrating establishment of protective immunity to an infectious pathogen in higher vertebrates by administration of genes encoding pathogen specific single chain FV antibodies. The protective activity of the pCDNA-BU1 construct fully correlated with the occurrence of neutralising anti-VHSV ScAbs in the plasma of injected fish, and although involvement of non-specific mechanisms cannot be completely excluded, it appears likely that the produced BU1 ScAb has been the major cause of protection following injection of the pCDNA3-BU1 plasmid DNA. Accordingly, in a later experiment including challenge of the fish with a virus isolate not recognised by the recombinant antibody fragment encoded by pCDNA-BU1, no protection was obtained.

In contrast to DNA-vaccines, including anti-idiotype vaccines, the administration of plasmid borne genes in this instance do

not involve specific activation of the immune system in the individual. The principle is simply that single chain FV antibody polypeptides produced by the cells that take up the administered plasmid will be systemically distributed by the body fluids and protect the individual if infection with the pathogen occurs. This corresponds to the mechanism of prophylaxis against infectious diseases in humans through administration of antiserum or immunoglobulin from immunised donors or animals, but without side effects such as risk of concomitant transfer of infectious diseases or induction of hypersensitivity following repeated administrations. In order to avoid the pathogen variability overcoming the immunity established by the plasmid, practical use may involve administration of plasmids encoding genes of single chain variable fragments to several different epitopes of the pathogen or single chain FV antibody gene-expression library towards a given pathogen.

The principle of genetic immunoprophylaxis according to the invention can be extended to mammals and to humans in particular as it is a valuable tool for transient protection of individuals such as travelers against exposure to pathogens or toxins where no efficient vaccines are available. Similarly, the invention may be used for induction of the synthesis of antibodies of a desired specificity for use in immunodeficient individuals. Also the nucleic acid construct of the present invention could be used in individuals that produce an allergic response to certain allergens, such as pollen. In this connection, production or induction of antibody fragments to those allergens may be used for prevention of an allergic reaction.

Beside the prophylactic aspects of the invention, plasmid constructs carrying genes encoding pathogen/disease antigen specific single chain FV antibodies are of therapeutic use in certain diseases wherein the host immune system itself is unable to produce antibodies with the necessary activity.

CLAIMS:-

1. A non-infectious nucleic acid construct encoding a recombinant antibody molecule, said construct being adapted for the *in vivo* establishment of a protective immunity to an infectious disease in an animal.

2. A non-infectious nucleic acid construct encoding a recombinant antibody molecule, said construct is formulated for the *in vivo* prevention of an allergic reaction to an allergen in an animal.

3. A non-infectious nucleic acid construct encoding a recombinant antibody molecule, wherein said construct is formulated for the *in vivo* prevention of a reaction caused by the presence of a toxic substance in an animal.

4. A construct according to claim 1 wherein the antibody molecule is derived from an antibody raised against the pathogen causing the disease.

5. A construct according to claim 2 wherein the antibody molecule is derived from an antibody raised against the allergen.

6. A construct according to claim 2 wherein the antibody molecule is derived from an antibody raised against IgE molecules.

7. A construct according to claim 3 wherein the antibody molecule is derived from antibodies raised against the toxic substance.

8. A construct according to claim 7 wherein the toxic substance is a venom or toxin produced by a poisonous organism.

9. A construct according to any preceding claim wherein the antibody molecule comprises variable domains of immunoglobulin Heavy and Light chain linked together by a linker sequence.

10. A construct according to any preceding claim further comprising a genetic sequence encoding secretion signal peptide.

11. A construct according to any preceding claim formulated for delivery by injection, spray or gene gun.

12. A construct according to any preceding claim comprising genes encoding an antibody molecule to several different epitopes on a given pathogen, allergen, or toxin.

13. A construct according to any preceding claim comprising genes encoding an FV antibody gene-expression library to a given pathogen, allergen, or toxin.

14. A construct according to any preceding claim including a viral haemorrhagic septicaemia virus VHSV-neutralising monoclonal antibody 3F1H10 with two amino acids substituents in the H-chain gene respectively Asn 35a to Thr and Lys 64 to Thr and with the secretion signal of rainbow trout transforming growth factor (TGF-beta) added to the 5′ end of the gene.

15. A method of treating an animal comprising administering thereto a construct as claimed in any of claims 1 to 14.

16. A pharmaceutical composition comprising a construct as claimed in any one of claims 1 to 14.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/03605

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/13 C07K16/08 C07K16/42 A61K39/395 A61P37/08
A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 25826 A (UNIVERSITY OF MANITOBA) 27 May 1999 (1999-05-27) claims 17-19 ---	1,2,5,12
X	BEARZOTTI MONIQUE ET AL: "Fish rhabdovirus cell entry is mediated by fibronectin." JOURNAL OF VIROLOGY, vol. 73, no. 9, 1999, pages 7703-7709, XP002157453 ISSN: 0022-538X the whole document ---	1,3,4, 15,16
X	US 5 614 611 A (CHANG) 25 March 1997 (1997-03-25) claims 1-7 --- -/--	2,6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 2001	22/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Flao, K

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication.where appropriate. of the relevant passages	Relevant to claim No.
A	NUNEZ E ET AL: "Phospholipid interactions of a peptide from the fusion-related domain of the glycoprotein of VHSV, a fish rhabdovirus." VIROLOGY, vol. 243, no. 2, 10 April 1998 (1998-04-10), pages 322-330, XP002157454 ISSN: 0042-6822 the whole document	1-16
A	HUANG CHIENJIN ET AL: "Mapping the neutralizing epitopes on the glycoprotein of infectious haematopoietic necrosis virus, a fish rhabdovirus." JOURNAL OF GENERAL VIROLOGY, vol. 77, no. 12, 1996, pages 3033-3040, XP002157455 ISSN: 0022-1317 the whole document	1-16
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Information on patent family members

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